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Vermiremoval of heavy metals from fly ash by employing earthworm *Eisenia foetida*

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ABSTRACT
Vermicomposting is commonly used for the management of organic wastes. A study was conducted to evaluate the efficiency of an exotic and epigeic earthworm species (*Eisenia foetida*) for bioaccumulation of heavy metals (Cr, Cu, Ni, Pb, Zn, Fe, Cd and Mn) during stabilization of fly ash amended with pressmud. The growth and reproduction of *E. fetida* was also monitored in a range of different feed mixtures for 60 days in laboratory under controlled experimental conditions. The heavy metals content in the different vermicomposts was lower than initial feed mixtures. Vermicomposted samples showed 25-45% reduction of heavy metals in 10-60% fly ash. The growth rate and cocoon production was maximum in 10% fly ash. Metal analysis of *E. foetida* revealed considerable bioaccumulation of different heavy metals in their body. The accumulation of Cr, Ni, Cd and Mn was maximum in 50% fly ash while Cu and Zn were in 10% fly ash in the earthworms body tissues. Pb and Fe were much accumulated by earthworms in 40% and 80% fly ash respectively. Performance of feed mixture with 10-60% fly ash was better than the other feed mixtures. The results indicated that fly ash could be converted into good quality manure by vermicomposting if mixed in an appropriate ratio (40-60%) with pressmud.
Keywords: Bioaccumulation, Eisenia foetida, Fly ash, Pressmud, Vermicomposting.

INTRODUCTION

Industrialization of the developing countries in the world of rapid economic growth has created serious problem of waste disposal due to rapid urbanization [1]. Fly ash is one of the major wastes produced by burning coal in thermal power stations. Its annual production all over the world is 300-350 million tonnes through combustion of 3000 million tonnes of coal [2, 3]. Fly ash contains silica, aluminium, oxides of iron, calcium, magnesium, arsenic, chromium, lead, zinc, nickel and other toxic heavy metals. Fly ash is a serious source of air pollution since it remains air borne for a long period of time and causes health hazards [4, 5]. Fly ash has been used as concrete additive and municipal land filler. The soil application of fly ash was also recommended to enrich the agricultural soil or to reclaim the sodic soil [6]. The use of fly ash in soil amendment raised a number of controversies. A study conducted, on crop grown in fly ash-amended soil showed improvement in plant growth and nutrient availability to crop plants [7, 8], on the contrary, other studies demonstrated heavy metal uptake by plants [9] which may lead to mass exposure of humans through food chain contamination and can cause serious health hazards [10, 11]. Considering the presence of toxic heavy metals in fly ash and their leaching in the environment [12], the reclamation of fly ash has become an important issue [13]. To overcome these adverse effect fly ash, a burnt material and therefore contains little organic matter, can be supplemented and vermicomposted by mixing with an additional source of organic matter like pressmud. Vermicomposting of organic waste has an important part to play in an integrated waste management strategy. Vermicomposting is basically composting with worms. In vermicomposting, worms are fed with decomposed matter and the organic material passes through the earthworm gut whereby a rich end product called worm castings is produced. A worm casting consists of organic matter that has undergone physical and chemical breakdown through the muscular gizzard, which grinds the material to a particle size of 1 to 2 microns. Nutrients present in worm castings are readily soluble in water for uptake by plants [14]. The earthworms accumulate metal within their tissue by binding with some metalloproteins called as metallothioneines. Generally, earthworms need to consume great amount of soil to achieve their daily nutrition, during which the digestive process liberates heavy metals in their free forms in the gut lumen. These freely available metals are then absorbed by the gut epithelial lining. This way, earthworms tend to accumulate considerable amount of the heavy metals within the cells in alimentary canal [15]. *Eisenia foetida* have to accumulate the heavy metals in their bodies from soil as well as different biological wastes during vermicomposting [16-19]. The *Eisenia foetida* and *Eudrilus eugeniae* are most important species for effective reducing of the metal toxicity from municipal solid wastes [20]. The main objectives of the present study were (i) to analyze the heavy metal status in fly ash and pressmud mixtures before and after vermicomposting, (ii) to analyze the concentration of heavy metals accumulated in earthworm tissues after vermicomposting and (iii) to analyze the biomass growth and cocoon production of earthworm *Eisenia foetida* in different feed mixtures after vermicomposting.

MATERIALS AND METHODS

Collection of materials

Fly ash was procured from the dumping ground of Panki Thermal Power Station near Panki, Kanpur,

India. Pressmud was procured from Kisan Sahkari Sugar Mill, Kayamganj, Farrukhabad, India. Earthworm species (*Eisenia foetida*) was procured from Kanpur Gaushala Society, Bhauti, Near Panki Highway, Kanpur, India and cow dung was procured from a cow farm near the university campus as a culturing material for earthworms.

Earthworms culture

The culture of earthworms (*Eisenia foetida*) was maintained under laboratory conditions by using cow dung as a culturing material. The worm's culture was needed for time to time use of earthworms for research work. Generally, *Eisenia foetida* survive on temperature range 16ºC – 28ºC and are most active on upper ends of its temperature range. In summer season worms enhance their foraging activities and are sexually more active. So the worm's culture was produced in the summer season.

Experimental design

Eleven feed mixtures having different proportions of fly ash and pressmud were established including one feed of 100% pressmud as control (Table 1). All the fly ash and pressmud quantities were used on dry weight basis that were obtained by drying known quantities of material at 110ºC to constant mass in a hot air oven. One kg of feed mixture was put in circular plastic containers (diameter 20 cm and depth 20 cm). All containers were kept in darkness at room temperature (22-26ºC). The moisture content of the feed mixture in each container was maintained at 60-80% throughout the study period by sprinkling adequate quantity of distilled water. These feed mixtures were turned manually every day for 21 days in order to eliminate volatile gases potentially toxic to earthworms. After 21 days, fifty nonclitellated hatchlings of *Eisenia foetida* of our own culture were introduced in each container. There were three replicates for each feed mixture and no additional food was added at any stage during the study period. After 60 days granular tea like vermicompost appear on the upper surface of each feed mixture excepting feed mixture no.1. The prepare vermicomposts and inoculated earthworms were used for analysis.

Heavy metals analysis

In initial feed mixtures and final vermicompost:

The samples were used for heavy metals analysis on a dry weight basis obtained by oven drying the known quantities of material at 110ºC. The heavy metals concentration in initial feed mixtures and final vermicompost were determined by using the method of Berman [21] by means of Atomic Absorption Spectrophotometer (Model: 220 FS, Varian, Australia) after digestion of the sample with concentrated Perchloric acid ($HClO_4$) and concentrated Nitric acid (HNO_3) (1:6, v/v).

In earthworms body:

For the analysis of heavy metals concentration in earthworms, the earthworms were rinsed free of sample particles and starved on moistened filter paper for 5 days to eliminate the organic and inorganic content of the alimentary canals. They were then oven dried at 65ºC for 4 days and crushed. All samples of known weights were wet digested in a mixture of Perchloric acid ($HClO_4$): Nirtic acid (HNO_3) (1:6 v/v) till a white residue remain at the bottom. This residue was then dissolve in 0.1N HNO3 [21]. The concentrations of Cr, Cu, Ni, Pb, Zn, Fe, Cd and Mn were determined with an Atomic Absorption Spectrophotometer (Model: 220 FS, Varian, Australia).

Growth study of earthworms

Earthworm growth parameters i.e. individual weight, earthworm weight gain, individual growth rate, cocoon production and juveniles production etc. were analyzed for growth study of *Eisenia foetia*. At the end of the vermicomposting period, the feed in the plastic bins were turned out. Earthworms, cocoons and juveniles were separated from the feed by hand sorting, after which they were counted and weighed after washing them with water and drying them by paper towels. Growth rate of earthworms was determined by using the method of Suthar [22].

All the chemicals used were analytical reagent (AR) grade supplied by Merck Limited, Mumbai. Alkali resistant borosilicate glass apparatus supplied by Borosil Glass Works Limited, Mumbai and double distilled water was used throughout the study for analytical work. All the samples were analyzed in triplicate and results were averaged. Homogenized samples of final vermicompost were stored in airtight plastic vials for further chemical analysis.

RESULTS

Heavy metals concentration in final vermicompost and in earthworms

Heavy metals appear in the Fly ash and Pressmud from a variety of sources like coal, soil and dust etc. So, the vermicompost made from fly ash and pressmud may have higher heavy metal concentrations. In small amounts, many of these elements may be essential for plant growth, however, in higher concentrations they are likely to have detrimental effects upon plant growth [23]. In the present study, initial heavy metal content of fly ash, pressmud and their different proportions were analyzed which resulted in higher heavy metal concentrations in initial feed mixtures (Table 2). A significance decrease in results showed that heavy metals, viz, Cr, Cu, Ni, Pb, Zn, Fe, Cd and Mn concentrations in final vermicompost in all the feed mixtures were lower than in the initial feed mixtures (Table 3). The concentration of chromium

(Cr) in the final vermicompost was remarkably reduced as compared to the initial feed mixtures. There was a reduction of 11.73% - 41.61% Cr in different feed mixtures by the end of vermicomposting period. Data revealed that Cr reduction was higher (41.61%) in feed mixture no. 10. Cr reduction was directly related to the pressmud content in the feed mixtures, i.e. the reduction was maximum for feed mixture no. 10 (41.61%) and minimum for feed mixture no. 1 (11.73%). A significant decrease in the concentration of copper (Cu) occurred following the vermiconversion of fly ash and pressmud into vermicompost in different vermireactors. The initial Cu concentration of the different mixtures was in the range of 78.60 mg/kg – 105.92 mg/kg (Table 2). The Cu concentration decreased in the range of 65.88 mg/kg – 78.79 mg/kg in different feed mixtures (Table 3) after vermicomposting. There was a reduction of 7.30% - 38.52% Cu in different feed mixtures by the end of vermicomposting period. Data revealed that Cu reduction was higher (38.52%) in feed mixture no. 10. A significance decrease in the concentration of nickel (Ni) occurred following the vermiconversion of fly ash and pressmud into vermicompost in different vermireactors. The initial Ni concentration of the different feed mixtures was in the range of 22.50 mg/kg – 88.21 mg/kg (Table 2). Ni decreased in the range of 14.51 mg/kg – 81.83 mg/kg (Table 3) in different feed mixtures after vermicomposting. There was a reduction of 7.71% - 37.11% Ni in different feed mixtures by the end of vermicomposting period. Data revealed that Ni reduction was higher (37.11%) in feed mixture no. 10. The initial concentration of lead (Pb) was in the range of 37.39 mg/kg – 54.93 mg/kg in different feed mixtures (Table 2). Final Pb concentration was decreased in the range of 19.08 mg/kg – 51.90 mg/kg (Table 3) in different feed mixtures after vermicomposting. There was a reduction of 7.30% - 47.98% Pb in different feed mixtures by the end of vermicomposting period. Data revealed that Pb reduction was higher (47.98%) in feed mixture no. 10. A significance decrease in the concentration of zinc (Zn) occurred following the vermiconversion of fly ash and pressmud into vermicompost in different vermireactors. The initial Zn concentration of the different feed mixtures was in the range of 83.74 mg/kg – 178.49 mg/kg (Table 2). Zn decreased in the range of 73.65 mg/kg – 96.09 mg/kg (Table 3) in different feed mixtures after vermicomposting. There was a reduction of 12.41% - 46.21% Zn in different feed mixtures by the end of vermicomposting period. Data revealed that Zn reduction was higher (46.21%) in feed mixture no. 10. The initial concentration of iron (Fe) was in the range of 431.50 mg/kg – 863.36 mg/kg in different feed mixtures (Table 2). Final Fe concentration was decreased in the range of 378.88 mg/kg – 529.12 mg/kg (Table 3) in different feed mixtures after vermicomposting. There was a reduction of 12.26% - 44.72% Fe in different feed mixtures by the end of vermicomposting period. Data revealed that Fe reduction was higher (44.72%) in feed mixture no. 10. The concentration of cadmium (Cd) in the final vermicompost was remarkably reduced as compared to the initial feed mixtures. There was a reduction of 10.14% - 47.93% Cd in different feed mixtures by the end of vermicomposting period. Data revealed that Cd reduction was higher (47.93%) in feed mixture no. 10. Cd reduction was directly related to the PM content in the feed mixtures, i.e. the reduction was maximum for feed mixture no. 10 (47.93%) and minimum for feed mixture no. 1 (10.14%). A significant decrease in the concentration of manganese (Mn) occurred following the vermiconversion of fly ash and pressmud into vermicompost in different vermireactors. The initial Mn concentration of the different mixtures was in the range of 92.21 mg/kg – 321.40 mg/kg (Table 2). The Mn concentration decreased in the range of 51.07 mg/kg – 288.24 mg/kg in different feed mixtures (Table 3) after vermicomposting. There was a reduction of 9.93% - 43.79% Mn in different feed mixtures by the end of vermicomposting period. Data revealed that Mn reduction was higher (43.79%) in feed mixture no. 10. Mn reduction was directly related to the pressmud content in the feed mixtures, i.e. the reduction was maximum for feed mixture no. 10 (43.79%) and minimum for feed mixture no. 1 (9.93%). Our findings are supported by Martin and Bullock [24] who reported a decrease in heavy metal concentration in vermicompost of oak wood. Similarly, Kumar et al., [25] have attributed the greater decrease in heavy metals in the castings, as opposed to in the municipal solid waste without earthworms, to the mineralization process that earthworms accelerate during municipal solid waste decomposition and stabilization.

Growth and cocoon production of *Eisenia foetida*

The biomass production by *E. foetida* in different feed mixtures has been encapsulated in Figure 1. The net weight gain by *E. foetida* was highest (776 mg earthworm^{-1}) in feed mixture no. 10 and lowest (364 mg earthworm^{-1}) in feed mixture no. 1. Increasing percentage of PM in the feed mixtures promoted the increase in biomass gain by *E. foetida*. The fastest growth rate (8.55 mg worm^{-1} day^{-1}) was observed in feed mixture no.10 (Figure 2) where as feed mixture no. 1 supported the least growth (1.18 mg worm^{-1} day^{-1}). The total number of cocoons after 60 days in different feed mixtures has been represented in Figure 3. The maximum no. of cocoons were observed in feed mixture no. 10 and minimum were in feed mixture no. 4. Similarly, the total numbers of juveniles were highest in feed ixture no.10 and lowest in feed mixture no. 4.

Table 1: Content (percentage) of Fly ash and Pressmud in initial feed mixtures

Feed Mixture No.	Fly Ash	Pressmud
1	1000[a] (100)[b]	-----
2	900[a] (90)[b]	100[a] (10)[b]
3	800[a] (80)[b]	200[a] (20)[b]
4	700[a] (70)[b]	300[a] (30)[b]
5	600[a] (60)[b]	400[a] (40)[b]
6	500[a] (50)[b]	500[a] (50)[b]
7	400[a] (40)[b]	600[a] (60)[b]
8	300[a] (30)[b]	700[a] (70)[b]
9	200[a] (20)[b]	800[a] (80)[b]
10	100[a] (10)[b]	900[a] (90)[b]
11	-----	1000[a] (100)[b]

a – The figures indicate the weight content in the initial feed mixtures (d/w).
b – The figures in parentheses indicate the percentage content in the initial feed mixtures.

Table 2: Concentration of Heavy metals (mg kg^{-1}) in initial feed mixtures

Feed mixture No.	Cr	Cu	Ni	Pb	Zn	Fe	Cd	Mn
1	123.48 ± 10.65	78.60 ± 5.80	88.21 ± 3.08	54.93 ± 4.10	83.74 ± 8.23	431.50 ± 14.65	43.89 ± 1.63	321.40 ± 13.16
2	109.30 ± 4.03	83.19 ± 3.43	79.13 ± 2.68	52.48 ± 5.28	95.41 ± 3.13	597.50 ± 18.50	37.85 ± 1.59	293.49 ± 20.84
3	101.20 ± 5.25	86.33 ± 3.26	71.62 ± 2.91	50.50 ± 2.05	106.92 ± 5.49	582.63 ± 20.37	33.20 ± 2.18	267.65 ± 21.48
4	92.70 ± 7.50	90.73 ± 4.23	63.91 ± 2.39	48.19 ± 2.16	117.43 ± 8.62	532.05 ± 32.34	29.23 ± 1.13	245.30 ± 12.05
5	82.90 ± 3.93	90.55 ± 3.51	58.64 ± 2.72	47.12 ± 2.02	127.94 ± 5.33	664.94 ± 21.91	23.68 ± 1.94	218.23 ± 13.02
6	71.70 ± 4.11	93.64 ± 2.97	51.80 ± 2.19	45.46 ± 3.38	137.28 ± 16.34	713.45 ± 25.66	19.25 ± 0.85	192.53 ± 7.48
7	61.10 ± 3.39	95.73 ± 3.70	43.47 ± 1.59	42.10 ± 2.35	146.96 ± 12.13	739.34 ± 31.78	16.10 ± 0.60	166.52 ± 10.63
8	52.90 ± 2.23	99.39 ± 4.15	38.96 ± 1.86	40.18 ± 1.96	158.47 ± 10.53	759.68 ± 41.11	11.85 ± 0.47	142.17 ± 7.24
9	40.50 ± 3.04	101.30 ± 4.14	30.49 ± 1.44	39.31 ± 1.63	168.98 ± 15.37	885.72 ± 33.63	6.65 ± 0.31	116.88 ± 6.26
10	32.60 ± 1.79	105.92 ± 3.33	22.50 ± 0.94	37.39 ± 2.81	178.49 ± 6.58	863.36 ± 30.97	3.19 ± 0.34	92.21 ± 4.32
11	28.43 ± 2.34	103.79 ± 3.91	19.64 ± 1.06	38.85 ± 3.93	185.96 ± 13.92	935.00 ± 41.79	BDL	73.72 ± 5.08

Values are means of three replicates ±; Standard deviation

Table 3: Concentration of Heavy metals (mg kg^{-1}) in final vermicompost

Feed mixture No.	Cr	Cu	Ni	Pb	Zn	Fe	Cd	Mn
1	109.62 ± 4.03	72.50 ± 3.06	81.83 ± 5.75	51.90 ± 3.73	73.65 ± 4.51	378.88 ± 13.71	39.90 ± 2.71	288.24 ± 12.31
2	96.59 ± 3.90	74.35 ± 2.73	71.52 ± 3.16	46.27 ± 1.78	81.96 ± 3.15	497.12 ± 25.90	33.44 ± 1.32	254.27 ± 11.57
3	83.92 ± 2.61	78.79 ± 3.12	61.40 ± 3.06	40.57 ± 2.72	84.48 ± 2.80	470.29 ± 17.66	27.55 ± 1.23	228.14 ± 15.81
4	73.28 ± 3.23	76.75 ± 3.17	49.16 ± 2.05	35.28 ± 1.66	91.28 ± 4.86	395.20 ± 14.04	23.80 ± 2.08	194.52 ± 12.95
5	63.40 ± 2.55	74.27 ± 4.29	42.81 ± 2.29	33.45 ± 1.62	92.41 ± 2.98	487.36 ± 21.04	18.22 ± 0.51	168.29 ± 10.14
6	50.84 ± 2.69	70.94 ± 2.01	35.67 ± 1.17	32.41 ± 3.27	92.23 ± 4.15	507.69 ± 18.28	14.35 ± 0.57	142.74 ± 8.86
7	42.45 ± 2.48	65.89 ± 2.13	29.35 ± 3.27	26.48 ± 0.98	92.21 ± 3.74	493.71 ± 24.67	10.61 ± 0.48	113.86 ± 11.78
8	32.28 ± 2.47	69.48 ± 3.43	26.01 ± 1.24	23.40 ± 1.92	95.61 ± 3.02	477.87 ± 15.96	7.08 ± 0.27	92.05 ± 8.54
9	24.45 ± 1.01	66.09 ± 3.03	18.75 ± 1.09	21.38 ± 0.98	95.26 ± 5.49	529.12 ± 29.77	3.22 ± 0.22	69.48 ± 5.41
10	19.86 ± 0.66	65.88 ± 2.31	14.51 ± 0.71	19.08 ± 0.91	96.09 ± 3.39	476.99 ± 22.24	1.56 ± 0.09	51.07 ± 6.50
11	15.38 ± 1.69	56.18 ± 2.68	12.69 ± 0.87	20.25 ± 0.77	94.49 ± 4.58	460.33 ± 19.90	BDL	37.63 ± 5.64

Values are means of three replicates ±; Standard deviation

Table 4: Concentration of heavy metals accumulated by earthworms (mg kg^{-1})

Feed mixture No.	Cr	Cu	Ni	Pb	Zn	Fe	Cd	Mn
1	15.29 ± 0.58	6.84 ± 0.23	6.80 ± 0.25	4.01 ± 0.26	10.40 ± 0.34	52.89 ± 1.87	4.45 ± 0.20	31.90 ± 1.02
2	15.52 ± 0.62	9.24 ± 0.28	7.73 ± 0.40	6.38 ± 0.21	14.27 ± 0.51	115.10 ± 4.02	4.94 ± 0.15	38.68 ± 2.06
3	20.61 ± 0.77	10.22 ± 0.30	10.42 ± 0.52	10.21 ± 0.32	25.47 ± 1.20	286.39 ± 11.72	5.53 ± 0.18	49.61 ± 1.71
4	29.03 ± 1.01	19.20 ± 0.89	14.49 ± 0.54	17.34 ± 0.54	42.78 ± 1.72	189.11 ± 9.07	8.98 ± 0.24	80.70 ± 2.96
5	31.72 ± 1.43	33.56 ± 1.15	22.05 ± 1.12	21.11 ± 0.94	57.37 ± 1.82	308.28 ± 10.47	10.36 ± 0.43	97.50 ± 4.07
6	23.04 ± 1.02	37.52 ± 1.51	20.99 ± 0.68	22.55 ± 0.71	66.50 ± 2.64	347.66 ± 10.15	9.05 ± 0.49	85.17 ± 2.89
7	25.25 ± 1.30	40.69 ± 1.32	18.42 ± 0.97	23.72 ± 0.82	76.30 ± 4.10	339.73 ± 11.52	8.52 ± 0.34	85.86 ± 4.34
8	22.84 ± 1.20	40.19 ± 1.02	13.77 ± 0.65	19.22 ± 0.89	74.70 ± 2.36	358.49 ± 11.81	6.17 ± 0.20	72.68 ± 2.73
9	19.44 ± 0.69	40.03 ± 2.13	11.60 ± 0.44	19.43 ± 0.62	73.17 ± 2.40	442.49 ± 10.70	4.18 ± 0.18	62.78 ± 3.03
10	16.13 ± 0.82	41.86 ± 1.88	8.35 ± 0.52	19.81 ± 0.74	87.85 ± 3.04	429.26 ± 14.58	1.88 ± 0.12	50.52 ± 1.90
11	14.53 ± 0.68	52.43 ± 1.71	7.51 ± 0.43	19.10 ± 0.66	78.92 ± 3.35	471.56 ± 22.60	0.72 ± 0.10	31.47 ± 1.14

Values are means of three replicates ±; Standard deviation

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Table 5: Percentage (%) reduction of heavy metals in different feed mixtures after vermicomposting

Feed mixture No.	Cr	Cu	Ni	Pb	Zn	Fe	Cd	Mn
1	11.73 ± 2.23	7.30 ± 2.34	7.71 ± 3.50	7.30 ± 3.59	12.41 ± 3.91	12.26 ± 3.12	10.14 ± 3.73	9.93 ± 2.09
2	12.03 ± 2.71	10.16 ± 4.13	9.77 ± 3.39	12.16 ± 4.15	14.95 ± 3.29	16.26 ± 3.09	11.05 ± 4.29	13.18 ± 3.11
3	17.30 ± 3.19	11.84 ± 3.79	14.54 ± 4.09	20.21 ± 4.10	20.82 ± 5.17	19.15 ± 3.50	16.65 ± 3.60	14.54 ± 3.04
4	20.32 ± 2.30	15.16 ± 4.70	22.67 ± 3.79	25.98 ± 3.50	22.43 ± 6.36	25.54 ± 5.07	20.72 ± 3.85	20.90 ± 4.91
5	23.09 ± 3.52	18.06 ± 3.90	27.60 ± 4.68	28.80 ± 3.29	27.84 ± 4.15	26.36 ± 3.30	23.75 ± 4.43	22.67 ± 3.97
6	29.08 ± 4.58	25.07 ± 3.19	30.52 ± 4.29	29.60 ± 4.52	32.44 ± 5.50	28.73 ± 3.58	27.01 ± 4.47	26.24 ± 3.89
7	35.15 ± 4.44	31.50 ± 3.87	32.37 ± 3.66	36.34 ± 4.59	36.92 ± 4.32	33.14 ± 4.30	32.92 ± 3.75	31.56 ± 6.40
8	37.85 ± 4.28	30.44 ± 4.19	35.34 ± 4.85	40.83 ± 4.90	39.14 ± 5.66	37.19 ± 5.41	36.07 ± 4.27	35.12 ± 5.09
9	40.49 ± 3.60	34.52 ± 3.09	38.05 ± 4.08	44.42 ± 3.17	43.30 ± 5.14	39.95 ± 3.83	42.86 ± 4.66	40.71 ± 4.38
10	41.61 ± 3.55	38.52 ± 3.17	37.11 ± 4.27	47.98 ± 5.59	46.21 ± 3.69	44.72 ± 3.58	47.93 ± 5.96	43.79 ± 4.70
11	43.79 ± 4.36	45.51 ± 3.65	38.23 ± 3.57	48.16 ± 4.92	49.43 ± 4.53	50.43 ± 4.46	0.72 ± 0.16	49.68 ± 5.95

Values are means of three replicates ±; Standard deviation

Table 6: Heavy metal limits (mg kg^{-1}) for compost in USA and European countries

Heavy metal	EU limit range	USA biosolids limit
Chromium	70 – 200	1200
Copper	70 – 600	1500
Cadmium	0.7 – 10	39
Mercury	0.7 – 10	17
Nickel	20 – 200	420
Lead	70 – 1000	300
Zinc	210 - 4000	2800

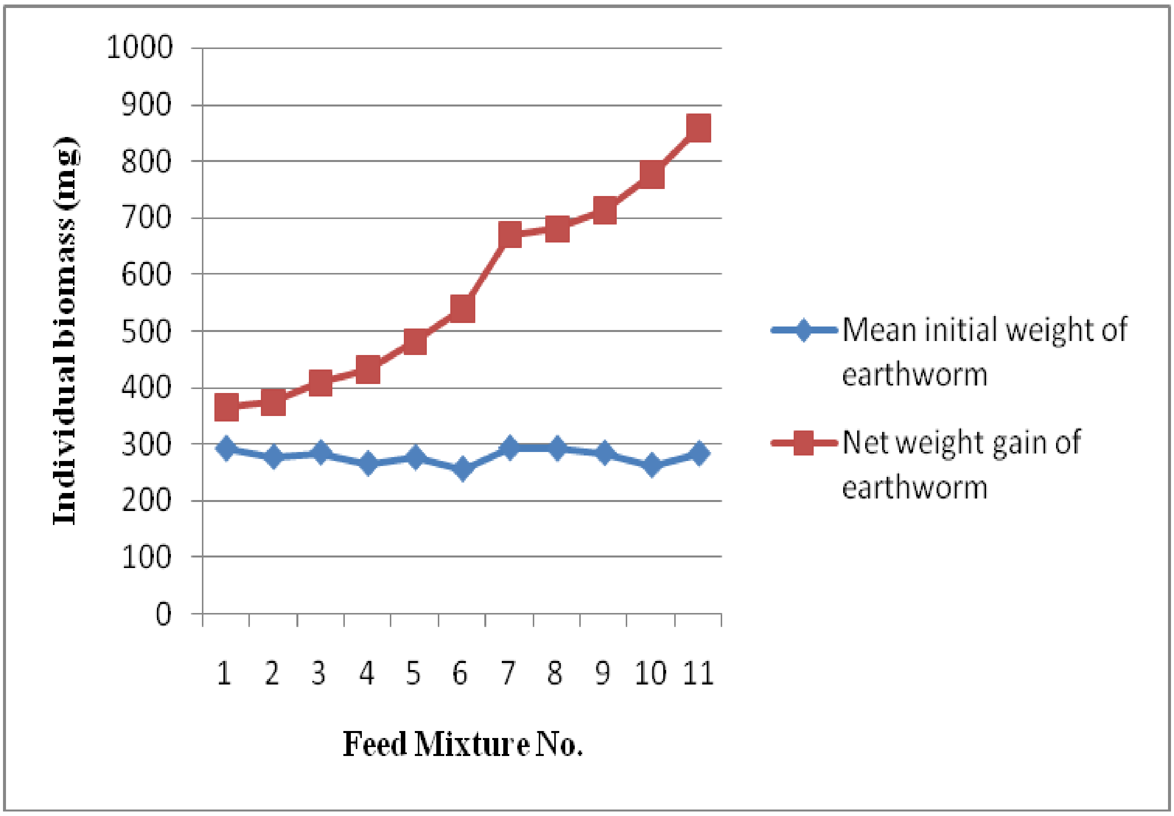

Figure 1: Growth curve of *Eisenia foetida* in different feed mixtures.

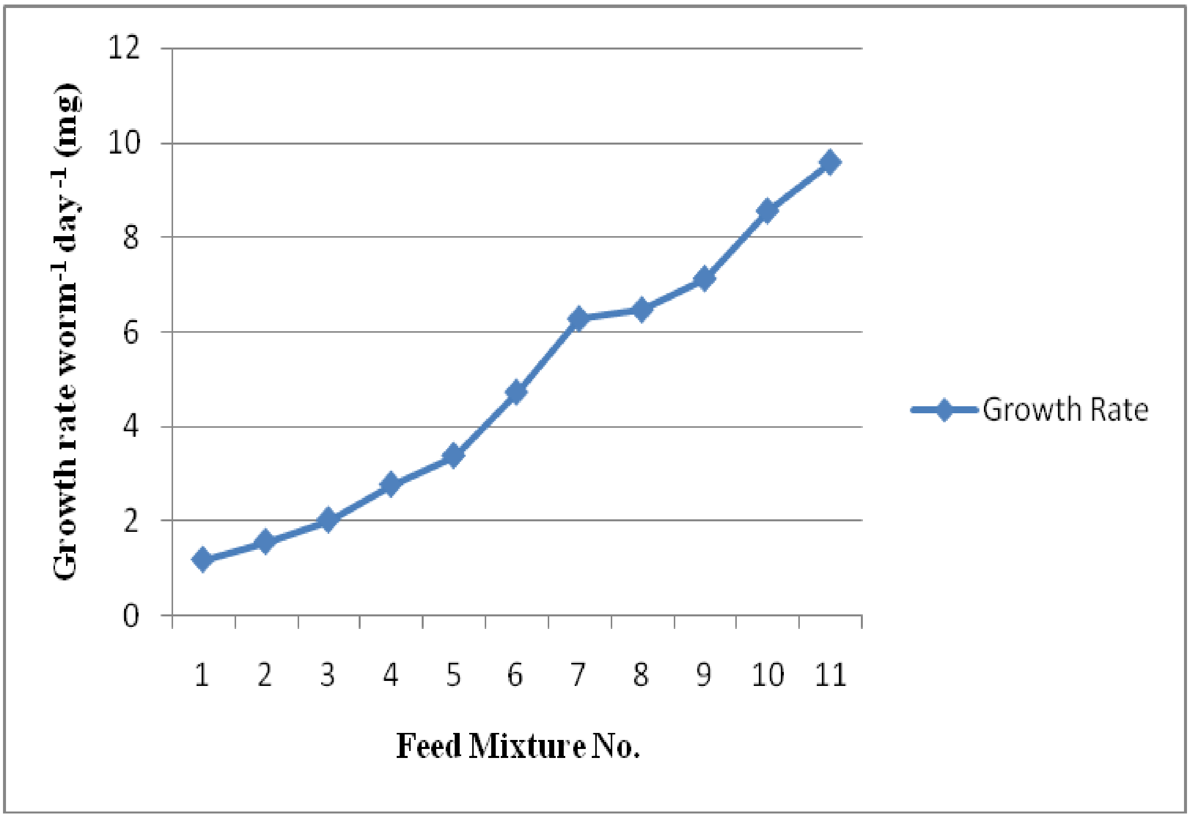

Figure 2: Growth rate of *Eisenia foetida* in different feed mixtures.

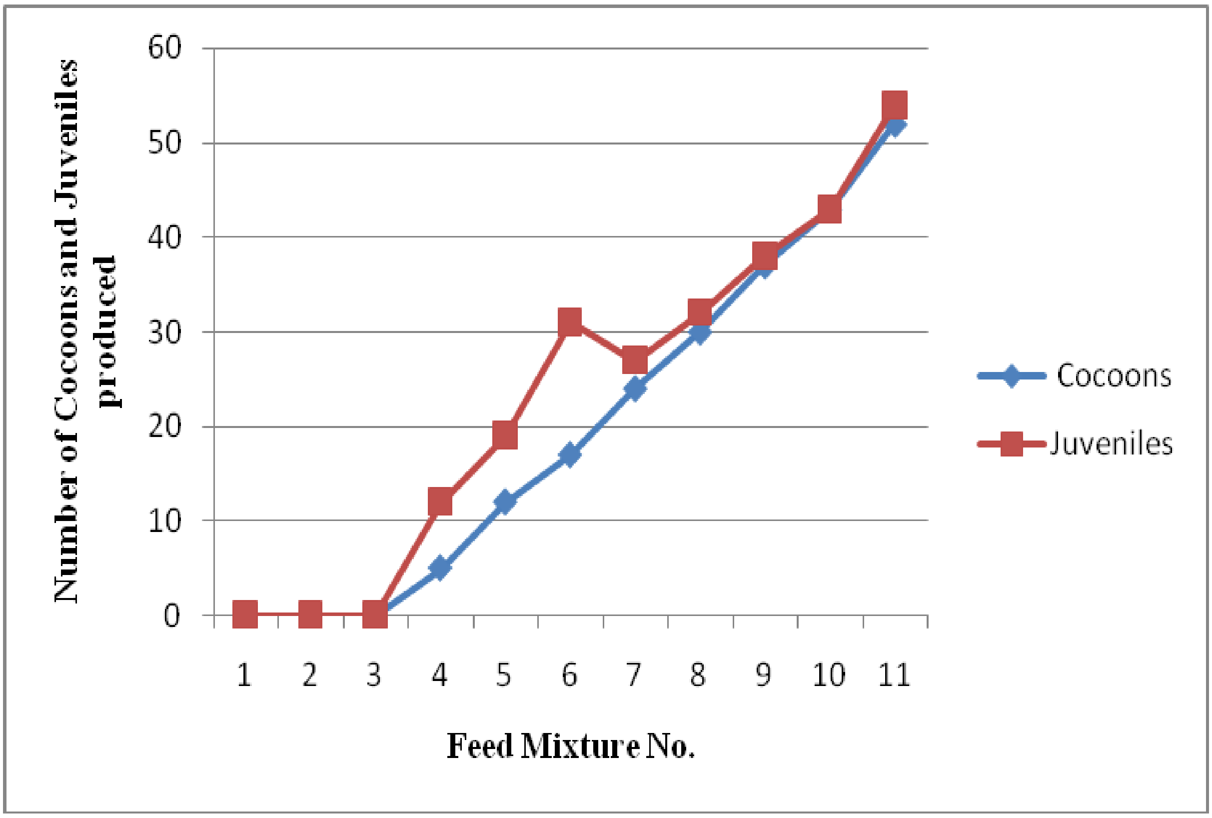

Figure 3: Total cocoons and juveniles produced by *Eisenia foetida* in different feed mixtures.

DISCUSSION

A significant amount of heavy metals was accumulated by earthworms *Eisenia foetida* in their body (Table 4). The data revealed that maximum accumulation of different heavy metals by earthworms was found in feed mixture no.5, 10, 9 and 7 respectively excepting control (feed mixture no. 11) and minimum in feed mixture no.1. Other workers have also reported similar observation [26, 27]. According to Wang et al., [28] earthworm species *Eisenia foetida* have ability to bioaccumulate heavy metals in their body tissues. According to Table 4 and 5, the heavy metals concentrations in the earthworm body tissues increased with the percentage of pressmud. In comparison of heavy metals content of initial feed mixture and inoculated earthworm body with respect to final vermicompost and earthworm body have a slit different may be due to the slit amount of heavy metals was accumulated by earthworms during vermiculture in cow dung prior to the experiment. Previous reports available on the metal accumulation ability of earthworms state that the metals like Cu and Ni are not bioaccumulated by the earthworms [29], but present results indicated considerable bioaccumulation of these metals in earthworms. While considering the risks associated with heavy metal contaminations in soils, it was found that the concentrations of heavy metals studied in the final vermicompost obtained from the different feed mixtures were lesser than limits set for composts in USA and European countries [30] (Table 6). *Eisenia foetida* could not tolerate the 100% fly ash. Addition of some other organic waste was essential for the survival of the earthworms in the fly ash. The growth rate expressed in terms of mg weight gained day^{-1} worm^{-1} has been considered as a good index to compare the growth of earthworms in different feeds [31]. It is evident from the figure 3 that the cocoons production was directly related to pressmud concentration in the studied feed mixtures. The results suggest that addition of fly ash in pressmud is not suitable for earthworm production (vermiculture) as the cocoon production is lesser if fly ash is present in the earthworm feed. Data revealed that number of cocoons and juveniles were zero in feed mixture no. 1,2 and 3. The difference between biomass and cocoon production in different feed mixtures could be related to the biochemical quality of the feed, which was one of the important factors in determining onset of cocoon production [32].

CONCLUSION

Disposal of fly ash by environmentally acceptable means is a serious problem. Our trials have demonstrated that vermicomposting can be an alternate technology for the management of fly ash mixed with pressmud. From the current study, we can safely conclude that the use of *E. foetida* to mitigate toxicity of metals seems to be feasible technology and up to 60% fly ash and 40% pressmud mixtures can be used for sustainable and efficient vermicomposting, without showing any toxicity to earthworms. The reduction of metal content was directly related to earthworm activity in the wastes decomposition system and the combination of pressmud with fly ash promotes the ability of accumulation of heavy metals in earthworm body. But addition of fly ash in the pressmud is not suggested if prime concern is vermiculture (production of earthworms) as the cocoon production is lesser if fly ash is present in the earthworm feed. Vermicomposting is set to become increasingly popular in this century as it yields rich organic fertilizers, recover energy rich resources, safety disposes organic wastes and helps tackle environment problem such as landfill and expense of collecting and transporting this waste. Vermicomposting waste will produce no pollution or unusable residue making it a very effective form of recycling. It is an ideal example as the worm composting process resemble closely to the nature. The environment is being damaged by human activities and we are also keen for saving the environment. Earthworms are perhaps nature's most ingenuous solution for a cleaner environment. However, further research on the effects of vermicomposting of such waste on the bioavailability of heavy metals should be carried out before utilizing this option for fly ash waste management. The study also inferred that the application of fly ash based vermicompost in the agricultural fields as a soil conditioner or manure, would not have any adverse effect due to heavy metals.

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